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Performance of a Highly Sensitive *Mycobacterium tuberculosis* Complex Real-Time PCR Assay for Diagnosis of Pulmonary Tuberculosis in a Low-Prevalence Setting: a Prospective Intervention Study

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ABSTRACT The potential impact of routine real-time PCR testing of respiratory specimens from patients with presumptive tuberculosis in terms of diagnostic accuracy and time to tuberculosis treatment inception in low-prevalence settings remains largely unexplored. We conducted a prospective intervention cohort study. Respiratory specimens from 1,020 patients were examined by acid-fast bacillus smear microscopy, tested by a real-time *Mycobacterium tuberculosis* complex PCR assay (Abbott RealTi*me* MTB PCR), and cultured in mycobacterial media. Seventeen patients tested positive by PCR (5 were acid-fast bacillus smear positive and 12 acid-fast bacillus smear negative), and *Mycobacterium tuberculosis* was recovered from cultures for 12 of them. Patients testing positive by PCR and negative by culture ($n = 5$) were treated and deemed to have responded to antituberculosis therapy. There were no PCR-negative/culture-positive cases, and none of the patients testing positive for nontuberculous mycobacteria ($n = 20$) yielded a positive PCR result. The data indicated that routine testing of respiratory specimens from patients with presumptive tuberculosis by the RealTi*me* MTB PCR assay improves the tuberculosis diagnostic yield and may reduce the time to antituberculosis treatment initiation. On the basis of our data, we propose a novel mycobacterial laboratory algorithm for tuberculosis diagnosis.

KEYWORDS acid-fast bacillus smear microscopy, mycobacterial culture, *Mycobacterium tuberculosis*, nontuberculous mycobacteria, real-time PCR

Rapid and accurate diagnosis of pulmonary tuberculosis (TB) leads to early initiation of targeted therapy resulting in improved patient outcomes and reduced transmission (1, 2). Nucleic acid amplification tests (NAAT) and, especially, real-time PCR assays allow the relatively sensitive and fast detection of *Mycobacterium tuberculosis* complex (MTC) in respiratory specimens, thus outperforming acid-fast bacillus (AFB) smear microscopy and conventional liquid- or solid-culture methods in terms of sensitivity and speed, respectively (3). Mathematical models exploring the economic impact of implementing NAAT in routine practice support the hypothesis that these methods are highly cost-effective for TB diagnosis regarding overall cost and quality-

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adjusted life years (4, 5). However, NAAT are not systematically included in routine TB diagnostic algorithms in many low-prevalence countries such as Spain (6, 7). Updated U.S. clinical practice guidelines for TB diagnosis in adults and children endorse the use of NAAT, albeit conditionally and based on low-quality evidence (8); uncertainties regarding field test performances and practical clinical utility have been invoked as accounting for this fact (6, 7). Nevertheless, a recent document issued by the Expert Opinion of the European Tuberculosis Laboratory Initiative Core Group Members for the WHO European Region (9) indicates that countries should prioritize the use of recommended rapid molecular tests, rather than conventional microscopy and culture, as the initial diagnostic test for adults and children presumed to have pulmonary TB.

Among NAAT, the FDA-approved Xpert MTB/RIF test (Cepheid, Sunnyvale, CA, USA) has been extensively evaluated (10), and the World Health Organization (WHO) endorses it as a replacement for smear microscopy in the initial diagnosis of TB in settings of high multidrug-resistant TB or HIV infection prevalence (11), where drug susceptibility testing is not usually performed. This assay performs suboptimally with AFB smear-negative specimens, with sensitivities of 45% to 77% (7, 10, 12–15). Abbott Molecular Inc. (Des Plaines, IL, USA) recently launched their automated RealTi*me* MTB (RT MTB) *in vitro* PCR assay for qualitative detection of MTC in respiratory specimens; it has a reported sensitivity of 77% to 96.7% in AFB smear-negative/culture-positive samples (16–23). The use of improved-sensitivity NAAT in such specimens in particular could have a major impact not only on the clinical and epidemiological management of TB but also on the diagnostic algorithms used in mycobacterial laboratories. We conducted a prospective nonrandomized intervention study to evaluate the analytical performance of the Abbott RealTi*me* MTB assay in a low-prevalence setting and to assess how using a routine operational diagnostic strategy based on universal screening of patients with presumptive TB with this PCR assay impacts the therapeutic management of TB.

MATERIALS AND METHODS

Study setting. The study was carried out at the Clínico-Malvarrosa health department, which covers a population of 344,000 inhabitants from the region northeast of Valencia and is linked to the 582-bed Hospital Clínico Universitario (HCU) tertiary referral and teaching hospital, and at the Gandía health department, which provides medical care to a population of 188,000 inhabitants and has a 411-bed hospital, the Francisco de Borja de Gandía hospital; both are in the Valencian Community, Spain. According to the Surveillance and Epidemiological Control Service of the Valencian Community, the rates of TB notification in 2016 at these health departments were 11.9 and 13.3 per 100,000 inhabitants, respectively.

Study population. Between 9 January and 9 May 2017, a total of 1,020 consecutive subjects over 18 years of age (median age, 77 years; range, 18 to 102 years) undergoing evaluation for pulmonary TB (869 patients from the Clínico-Malvarrosa health department and 151 from the Gandía health department) were enrolled in the study. No exclusion criteria were established. Patients with presumptive TB were managed according to a Spanish consensus document (24). This study was approved by the Ethics Committee at the Hospital Clínico Universitario Fundación INCLIVA, in January 2017. The need to obtain informed consent was waived because the Abbott PCR assay was included in our laboratory test menu prior to the initiation of this study.

Laboratory procedures. Respiratory specimens were screened for the presence of AFB by direct (nondecontaminated/nonconcentrated) microscopy using auramine-thiazine red staining. Positive microscopy results were confirmed using Ziehl-Neelsen staining. The specimens were then decontaminated by the use of a BBL MycoPrep kit (Becton Dickinson, Sparks, MD, USA), which employs N-acetyl-L-cysteine, sodium citrate, and NaOH at 2% (final concentration), as indicated by the manufacturer, and neutralized with phosphate buffer (67 mM; pH 6.8), followed by centrifugation at $3,000 \times g$ for 20 min. Sediments were resuspended in 3 ml of sterile phosphate-buffered saline (PBS), inoculated into a Löwenstein-Jensen (LJ) tube (0.5 ml) and a Bactec MGIT 960 tube (0.2 ml) (Becton Dickinson, Sparks, MD, USA), and incubated at 37°C for 3 months and at 35°C for 8 weeks, respectively (19, 25). Identification of MTC isolates was performed by the use of a SD TB Ag MPT 64 Rapid Immunochromatographic test (Standard Diagnostics, Seoul, South Korea). Differentiation of MTC members was achieved by using a GenoType MTC VER 1.X system (Hain Lifescience, Hardwiesenstraße, Germany). Species identification of nontuberculous mycobacteria (NTM) was performed by the use of a GenoType Mycobacterium CM or AS system (Hain Lifescience) or by 16S rRNA gene sequence analysis, as previously reported (19). AFB smears were performed at the respective local laboratories, whereas culture and identification were performed at HCU.

Real-time MTB PCR assay. The Abbott Real Ti*me* MTB assay allows the detection of 8 species belonging to MTC (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. caprae*, *M. canetti*, *M. microti*, and *M. pinnipedii*) through the amplification of both the IS*6110* insertion sequence and the protein

TABLE 1 Type of specimens and number of specimens/patient in the prospective cohort

Parameter	No. of specimens
Type of specimen	
Sputum	989
Bronchial aspirate[a]	310
Bronchoalveolar lavage fluid	91
Tracheal aspirate	17
Induced sputum	2
No. of specimens/patient	
One	735
Two	195
Three	76
Four	14

[a]Bronchial aspirate specimens were obtained by aspiration of bronchial content after bronchoscope insertion. A flexible bronchoscope was moved through the airways to a target lesion for sampling.

antigen B (PAB) gene. The assay was performed as previously reported (19). Briefly, a volume of 1.2 ml of each decontaminated and concentrated sediment sample was used for DNA extraction, which was performed using Abbott mSample Preparation System DNA (designed to use magnetic microparticle processes for the purification of DNA) and an Abbott m2000sp platform (Abbott Molecular Inc., Des Plaines, IL, USA). Amplification, MTC detection, and data interpretation were performed on an Abbott m2000rt real-time PCR instrument. Overall, the first specimen collected from each patient was run for real-time PCR detection of MTC DNA. Pooled samples were run for PCR when two or more specimens from the same patient were available prior to batch testing.

Diagnostic algorithms, intervention, and endpoints. The attending physicians were alerted by telephone of any positive AFB smear results as soon as they were available, and anti-TB treatment was initiated if deemed convenient at the discretion of the physician. PCR was performed in batches, three times a week (usually Monday, Wednesday, and Friday mornings); patient specimens were stored at 4°C for a maximum of 4 days prior to PCR testing. A member of our laboratory notified the attending physicians of any positive PCR results by telephone as soon as they became available, and that physician was responsible for locating the patient and initiating anti-TB treatment within 48 h. All specimens were cultured on MGIT and LJ media; when culture results were available, an alert (which attending physicians could access) was activated on the laboratory information system (LIS). TB patients were scheduled for clinical examination 2, 8, 16, and 24 weeks after the initiation of anti-TB therapy.

The main objective of this study was to assess the analytical performance of the Abbott RealTi*me* MTB PCR assay in clinical practice in individuals with presumptive pulmonary TB compared with that of a composite reference standard consisting of mycobacterial culture results and clinical TB treatment responses (in patients with culture/PCR result discordances). A response to TB therapy was defined as chest X-ray improvement and/or symptomatic improvement (weight gain, fever resolution, and cough or hemoptysis remission) (26), as judged by an independent expert panel of pulmonologists. Negative AFB smear conversion results or *M. tuberculosis* cultures in two consecutive respiratory specimens after 8 weeks of treatment also indicated adequate treatment response (26). A standardized case record form was built to collect demographic and clinical data (including comorbidities) and chest X-ray findings.

Statistical methods. Sample size calculation was deemed unfeasible owing to the fact that insufficient data on field analytical performance of the Abbott RealTi*me* MTB PCR assay are available in the literature. As stated above, this was a primary endpoint of the current study. The medians and means were compared using the Mann-Whitney U test and the Student *t* test, respectively; P values of $\leq$0.05 were deemed to be statistically significant.

Laboratory cost data. The following items were considered for laboratory cost analyses: MGIT vials, LJ medium tubes and associated expendables, SD Bioline TB Ag MPT64 Rapid immunochromatographic tests, nucleic acid DNA extraction procedures, RealTi*me* MTB PCR assays and related consumables, and laboratory labor. The prices of consumables were provided by the manufacturers, and the average hourly wage of the laboratory technicians/nurses/staff was reported by the Human Resources Department. Costs of specimen decontamination and concentration, AFB smear microscopy, MTC species discrimination, and NTM identification were excluded from the analyses, as explained in the Discussion below.

RESULTS

Pulmonary tuberculosis in the cohort. In all, 1,409 respiratory specimens (Table 1) from 1,020 consecutive subjects (Table 2) undergoing evaluation for pulmonary TB were received at HCU for routine mycobacterial diagnostic testing. Eighty-seven patients had a history of previous TB, but none had been treated with anti-TB drugs in the year prior to enrollment. *M. tuberculosis* was isolated on MGIT and LJ cultures from 27 specimens from 12 patients (1.1%), 5 (41.6%) of whom had a positive AFB smear result. Overall, the median time to positive MGIT culture was 11 days (range, 7 to 16 days). The median diagnostic times were 8 days (range, 7 to 11 days) for AFB smear-positive

TABLE 2 Demographics and baseline clinical characteristics of patients with or without tuberculosis in the prospective cohort

Characteristic	No. (%) of patients	
	TB (n = 17)[a]	Non-TB (n = 1,003)
Male sex	11 (64.7)	619 (61.7)
Symptoms		
Fever	3 (17.6)	195 (19.4)
Cough	12 (70.6)	532 (53)
Hemoptysis	5 (29.4)	145 (14.5)
Dyspnea	8 (47.1)	420 (41.9)
Weight loss	4 (23.5)	62 (6.2)
Hospitalization	12 (70.6)	450 (44.9)
Pathological chest radiography	15 (88.2)	542 (54)
Cavities	10 (58.8)	15 (1.5)
Previous tuberculosis history	3 (17.6)	84 (8.4)
Comorbidity conditions		
Diabetes mellitus	3 (17.6)	225 (22.4)
Malignancy	1 (5.9)	131 (13.1)
Chronic obstructive pulmonary disease	2 (11.8)	233 (23.2)
Bronchiectasis	2 (11.8)	161 (16)
Human immunodeficiency virus infection	2 (of 6 tested; 33.3)	23 (of 312 tested; 7.4)
Ever smoked	12 (70.6)	402 (40.1)
Alcoholism	4 (23.5)	66 (6.6)

[a]Patients PCR positive/culture positive for MTC and those PCR positive for MTC/culture negative. TB, tuberculosis.

patients and 12 days (range 8 to 16 days) for AFB smear-negative patients. Twenty patients tested positive for NTM (data not shown), and 7 of those patients were deemed to require antimicrobial treatment by the attending physician.

Performance of the Abbott RealTi*me Mycobacterium tuberculosis* complex PCR assay in patients with presumptive pulmonary tuberculosis. Seventeen of 1,020 patients tested positive by PCR (Table 3); 5 of those patients were AFB smear positive, and 12 were AFB smear negative. Among those 17 patients, 12 tested positive by culture. The remaining 5 patients tested negative in mycobacterial cultures and AFB smears. The mean PCR cycle threshold (C_T) value was significantly lower (P = 0.003) for culture-positive specimens (C_T = 30.65; range, 23.56 to 37.84) than for culture-negative samples (C_T = 36.56; range, 32.38 to 39.32). All PCR-positive/culture-negative patients were treated with anti-TB drugs and deemed to have responded to anti-TB therapy after 8 weeks of treatment; a final evaluation at 6 months after therapy initiation confirmed clinical and radiological response in 4 patients. Clinical and radiological worsening was documented in the remaining patient (currently undergoing therapy with a new anti-TB drug combination). These 5 cases were thus considered true positives for PCR and false negatives for culture; there were no PCR-negative/culture-positive cases, and no NTM-positive patients had a positive PCR test result. Hence, the overall sensitivity, specificity, negative predictive value, and positive predictive value of the PCR assay were 100% (95% confidence interval [CI], 81.6% to 100%), 100% (95% CI, 99.6% to 100%), 100% (95% CI, 99.6% to 100%), and 100% (95% CI, 81.6% to 100%), respectively, and those of culture were 70.6% (95% CI, 46.9% to 86.7%), 100% (95% CI, 99.6% to 100%), 100% (95% CI, 75.7% to 100%), and 99.5% (95% CI, 98.8% to 100%). For calculations of the predictive values, the prevalence of TB in the cohort was considered. Overall, the median time to TB diagnosis was 2 days (range, 1 to 6 days) following a testing schedule of three times/week. The overall time to targeted (anti-*M. tuberculosis*)

TABLE 3 Demographic, clinical, and microbiological data for patients who tested positive with the Abbott Real-Time MTB PCR assay[a]

Patient	Age (yrs)	Sex	Comorbidity(ies)	Previous TB	Hospitalization	Specimen(s) processed for PCR analysis (no.)	AFB	Culture	TP (days)	PCR C_T
MTB01	32	M	No	No	No	S (2)	Pos	Pos	7	26.26
MTB02	31	M	HIV	No	Yes	S (1)	Pos	Pos	11	33.64
MTB03	50	M	No	No	Yes	S (1)	Pos	Pos	8	23.56
MTB04	37	F	No	No	Yes	S (2)	Pos	Pos	9	28.10
MTB05	51	M	DM	No	Yes	S (3)	Pos	Pos	7	26.45
MTB06	68	M	No	No	Yes	TA (1)	Neg	Pos	12	31.42
MTB07	33	M	No	No	No	S (2)	Neg	Pos	8	35.32
MTB08	22	F	No	No	No	S (1)	Neg	Pos	11	32.71
MTB09	51	M	No	No	Yes	BA (1)	Neg	Pos	14	32.52
MTB10	50	M	HIV	Yes (May 1999)	Yes	BA/BAL (1)	Neg	Pos	14	33.61
MTB11	55	M	DM	No	No	S (2)	Neg	Pos	12	26.46
MTB12	33	M	No	No	Yes	S (1)	Neg	Pos	16	37.84
MTB13	62	F	BE	Yes (January 2015)	Yes	S (1)	Neg	Neg	NA	32.38
MTB14	46	M	No	No	No	S (1)	Neg	Neg	NA	38.23
MTB15	42	F	BE	No	No	S (2)	Neg	Neg	NA	35.30
MTB16	63	F	DM, MA, COPD	No	Yes	BA/BAL (1)	Neg	Neg	NA	37.61
MTB17	39	F	COPD	Yes (April 2006)	Yes	S (2)	Neg	Neg	NA	39.32

[a]AFB, acid-fast bacilli smear microscopy; BA, bronchial aspirate following targeted flexible bronchoscopy; BAL, bronchoalveolar lavage fluid; BE, bronchiectasis; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; F, female; HIV, human immunodeficiency virus type 1 infection; M, male; MA, malignancy; NA, not available; Neg, negative; PCR C_T, PCR cycle threshold; Pos, positive; S, sputum; TB, tuberculosis; TP, time of positivity of mycobacterial culture.

treatment initiation was 2 days (range, −4 to 6 days), and the times were 1 day (range, 0 to 3 days) for AFB smear-positive patients and 3 days (range, −4 to 6 days) for AFB-negative patients. One HIV-1-positive patient was treated based on clinical and radiological criteria before confirmation of the PCR result (day −4).

Performance of the Abbott RealTi*me* PCR assay in patients treated for tuberculosis. Follow-up sputum samples obtained after 8 weeks of treatment were submitted from 7 patients. The remaining 10 patients failed to produce a sample at the scheduled visit. Mycobacterial culture was negative for all 7 patients, whereas 2 patients tested positive by PCR (Table 4).

Laboratory cost. The costs assigned to evaluable items are shown in Table 5. The actual laboratory cost incurred by the conventional TB diagnostics flow during the study period was approximately €18,276. The use of the Abbott RealTi*me* MTC assay in addition to mycobacterial culture resulted in an extra cost of roughly €23,633 (€23.17 per single or pooled specimen).

DISCUSSION

The WHO and the U.S. FDA endorse the use of NAAT in patients with presumptive TB in countries with low TB prevalence (8, 9, 11, 26, 27); nevertheless, NAAT have not been systematically incorporated into routine TB diagnostic algorithms in these settings, most likely because of their relative insensitivity with AFB smear-negative specimens (9). Here, we show for the first time that routine screening of respiratory

TABLE 4 Clinical data and chest X-ray findings from patients testing positive by the Abbott Real-Time PCR and negative by mycobacterial culture[a]

Patient	Symptom(s)	Chest radiography suggestive of TB	Cavities	Treatment (mos)	Clinical evolution[b]	Radiological evolution	Follow-up sample[c]
MTB13	Co, Dys	Yes	No	HRZE (2)/HR (4)	Improvement	No major changes	Culture−/PCR+
MTB14	He	No	No	HRZE (2)/HR (4)	Improvement	Improvement	NA
MTB15	F, Co, He, Wl	No	No	HRZE (2)/HR (4)	Improvement	No major changes	Culture−/PCR−
MTB16	Co	Yes	No	HZE (12)	Improvement	Improvement	NA
MTB17	Co, He, Dys, Wl	Yes	Yes	Other[d]	Improvement	No major changes	NA

[a]Abbreviations: Co, cough; Dys, dyspnea; E, ethambutol; H, isoniazid; He, hemoptysis; F, fever; NA, not available; R, rifampin; Z, pyrazinamide; Wl, weight loss.
[b]Fever, hemoptysis, or cough remission and weight gain.
[c]After 8 weeks of therapy.
[d]This patient had had previous multidrug-resistant tuberculosis (resistance to isoniazid, rifampin, pyrazinamide, and streptomycin documented by means of phenotypic methods) and was treated with ciprofloxacin, ethambutol, linezolid, and azithromycin.

TABLE 5 Data used for analyses of laboratory costs

Item	Assigned cost (€)[a]
Conventional diagnostic strategy	
Bactec MGIT Mycobacteria Growth Indicator Tubes—BD and Panta (antibiotic mixture)	11.20/unit
Löwenstein-Jensen tubes	0.34/unit
Culture expendables	0.26/unit/specimen
SD TB Ag MPT64 Rapid Immunochromatographic (confirmatory) test	9.46/unit
Personnel	1.08/specimen[b]
Cost/specimen	12.89/negative MTC specimen and 22.35/positive MTC specimen
Total (during the study period)[c]	18,276
Abbott Real-Time MTB PCR strategy	
Abbott Real-Time MTB PCR (nucleic acid extraction + PCR analysis)	21.10/specimen (pooled or single specimens)
PCR expendables	1.80/specimen
Personnel (PCR testing)	0.27/specimen[d]
Cost/specimen	23.17
Total (during the study period)[e]	23,966

[a]The prices of consumables were provided by the manufacturers, and the average hourly wage of laboratory technicians/nurses/staff was reported by the Human Resources Department at the Hospital Clínico Universitario.
[b]The personnel cost has been calculated based on the time spent inoculating LJ tubes and MGIT bottles and monitoring for growth on LJ tubes (once a week during 3 months). According to our estimations, the time spent per specimen was 4 min.
[c]Total calculated for 1,409 cultured samples and 12 MTC confirmatory tests.
[d]The personnel cost has been calculated based on the time spent assembling the Abbott m2000 system and adjusted to an average of 25 samples per run (approximately 1 min/specimen).
[e]Total calculated for 1,020 samples, 17 LJ/MGIT cultures, and 12 MTC confirmatory tests.

specimens from individuals with presumptive TB, using a highly sensitive real-time PCR (the Abbott Real-Ti*me* MTC PCR assay) in a population with low pretest prevalence, increases the TB diagnostic yield compared to mycobacterial culture and leads to an overall reduction in the time to TB diagnosis, in particular, in AFB smear-negative patients. The exquisite analytical sensitivity of the Abbott PCR assay (limit of detection of 2.45 CFU/ml using the H37rv strain spiked into AFB-negative sputum samples [16]), which appeared to exceed those of the Xpert MTB/RIF test (131 CFU/ml) (10, 14) and mycobacterial cultures (nearly 10 CFU/ml) (28), likely accounted for the findings described above.

Five patients tested positive by PCR and negative by culture, but, on the basis of their chest X-ray signs and/or a clinical picture and response to TB treatment, it was concluded that all of them had TB. Moreover, none of them had had TB in the year before enrollment, which reasonably ruled out the possibility of false-positive PCR results caused by the presence of persistent bacterial DNA from nonviable microorganisms after successful TB treatment (29). Moreover, the PCR C_T values for PCR-positive/culture-negative specimens were in the upper quartile range, which clearly indicated that these samples contained small amounts of MTC DNA.

M. tuberculosis DNA or cDNA, probably from viable bacteria, has been detected in culture-negative respiratory specimens from patients with or without an objective and consistent clinical suspicion of pulmonary TB (30), hence supporting the assumption that culture is not 100% sensitive and, strikingly, suggesting that *M. tuberculosis* DNA may be detectable in individuals with latent infection. In this respect, no patient in our prospective cohort with a positive tuberculin or interferon-gamma-release-assay (IGRA) test result and a TB alternative diagnosis ($n = 21$) tested positive by PCR (not shown).

Two of 7 patients with available follow-up specimens tested positive by PCR after 8 weeks of treatment but yielded negative culture results. Those two patients displayed clinical and radiographic responses to anti-TB therapy. Since nonviable bacteria are a possible source of the MTC DNA detected in these patients (29), highly sensitive PCR assays may not be appropriate for controlling the efficacy of TB treatments. As an aside, a recent study performed in a community health center with a high incidence of TB and HIV coinfection (31) reported a sensitivity for raw sputum samples of 82.5% for the Abbott RealTi*me* PCR assay. Another very recent study (32), in this case, with a prospective design, that was also conducted in a region with a high incidence of TB infection reported overall sensitivity and specificity of 95.2% and 99.8%; methodological differences with respect to sample inactivation, dissimilarities in the clinical characteristics of the patients (especially in terms of HIV-1 coinfection), and the choice of the "gold standard" for comparative analyses (culture in these studies versus a composite of culture and clinical outcome following instauration of anti-TB therapy in ours) may explain this discrepancy.

As expected, overall, the time to TB diagnosis was dramatically shortened using the PCR assay, which led to prompt anti-TB treatment initiation, in particular, for AFB-smear negative patients. In effect, in our cohort, the median time to targeted (and appropriate) treatment initiation in this subset of patients was 3 days, but it would have been a median of 12 days, at best, had we relied only on MGIT culture results. This is of paramount relevance, since AFB smear-negative patients can transmit TB (33).

Following local guidelines, patients who likely have TB (but may not) are immediately treated upon assessment of positive AFB smear microscopy results. Nevertheless, the fact that anti-TB treatment may be erroneously begun on the basis of AFB smear-positive results in cases of AFB smear-positive lung NTM disease (two cases in our cohort) should be highlighted. Thus, a tangible advantage of the use of the Abbott real-time MTB PCR assay on AFB-positive smear specimens is that a negative test result would safely spare these patients from anti-TB treatment (given the very high negative predictive value of the PCR assay).

Current TB diagnostic algorithms generate a heavy workload for microbiology laboratory technicians and staff, which is highly unfruitful in settings of low TB prevalence such as ours. Given the excellent sensitivity and specificity of the Abbott Real-Ti*me* MTB PCR assay reported here, as well as the very low rate of assay inhibition (in the current study, PCR inhibition occurred in only 2 specimens from two patients who had an additional sample tested for the presence of MTC DNA), we suggest that a diagnostic strategy whereby only AFB smear-positive specimens and PCR-positive pooled samples are processed for mycobacterial culture (Fig. 1) would be both feasible and reasonable, provided, naturally, that our results are confirmed in further studies that include more TB cases. The use of pooled specimens (two or more) instead of single samples might increase the diagnostic yield (although such was not proven in the current study), given the discontinuous shedding of MTC (in sputum samples), and is cost saving. In our study, performing three runs/week allowed us to pool at least two sputum samples from most of patients who provided them.

Our strategy differs from that recently endorsed by the European Office of WHO (9), based upon the use of the Xpert MTB/RIF test, in that PCR testing is not implemented as an add-on procedure for all specimens. It is uncertain whether the advent of the novel Xpert MTB/RIF Ultra assay, whose sensitivity largely exceeds that of the former Xpert test for AFB-negative specimens and is seemingly comparable to that of the Abbott MTB PCR assay (34, 35), may lead the European Office of WHO to modify its strategy and recommend the use of the novel Xpert test in the manner that we propose for the Abbott MTB PCR test.

Our novel diagnostic approach would incur in an extra cost of approximately €5,690 (calculated for 1,409 specimens) compared to the conventional diagnostic algorithm; this estimation excludes the costs associated with decontamination-concentration procedures, AFB smear microscopy, and MTC species discrimination (common to both strategies) and that of NTM identification (because our proposed algorithm would make it impossible to quantify the NTM cases). Of note, this extra cost would have been substantially reduced or even eliminated had the recommended number of sputum specimens for TB diagnosis (no less than two and preferably three) been provided in all the cases in our cohort (Table 1). In any case, this amount would be insignificant if the cost savings from implementing routine PCR testing in TB diagnostic algorithms

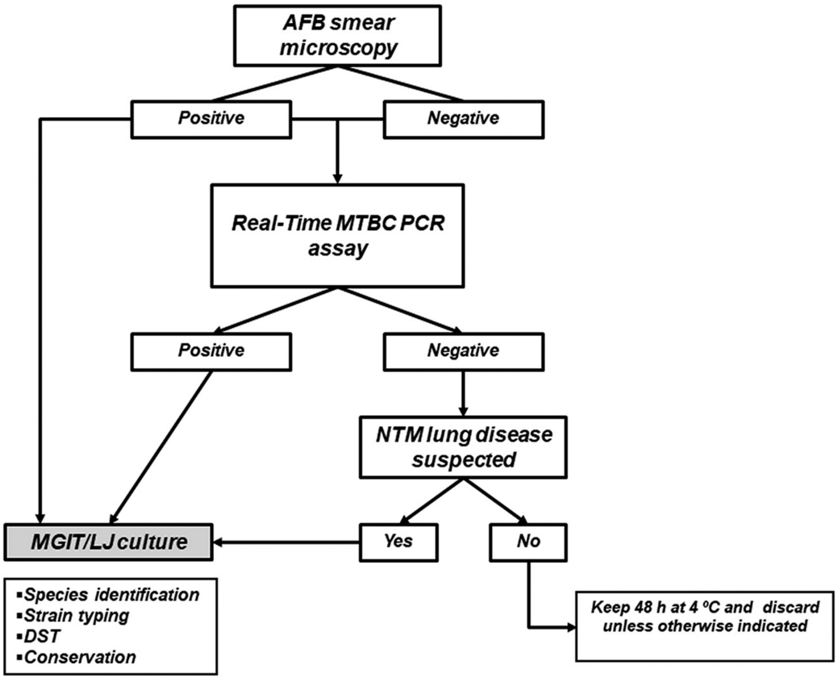

FIG 1 Diagram of the proposed laboratory workflow strategy for the diagnosis of tuberculosis and nontuberculous mycobacterial lung infections. Respiratory specimens from patients with suspected TB are first examined by AFB smear microscopy. AFB smear-positive specimens are processed for mycobacterial culture. All specimens (irrespective of AFB smear results) are tested by the Abbott real-time PCR assay (or any other NAAT assay with equivalent sensitivity). Positive MTC PCR specimens are cultured on Löwenstein-Jensen medium slant LJ and MGIT (Mycobacterium growth indicator tube) to confirm the infecting species strain conservation and typing and phenotypic drug susceptibility testing (DST) results. Negative PCR specimens are discarded unless derived from patients with a high level of clinical and radiological suspicion of NTM lung disease (targeted laboratory test ordering).

predicted by modeling studies are confirmed (4, 5, 14, 15, 36–38). Regarding NTM recovery, the incidence of clinically significant NTM lung disease is low in most high-income countries (39, 40) and its risk factors in adults have been reasonably delineated (39–43). In this novel approach, only specimens from patients with high-level suspicion of lung NTM disease would be processed for culture.

An obvious limitation of the Abbott RealTi*me* MTB PCR assay is its inability to provide any information on *M. tuberculosis* genotypic drug resistance, which is deemed useful even in settings of low TB prevalence. Nevertheless, Abbott Diagnostics has recently launched a RealTi*me* PCR MTB RIF/INH resistance assay which provides qualitative resistance detection within a single assay for the two most important first-line anti-TB drugs (rifampin and isoniazid). Thus, the Abbott RealTi*me* MTB PCR assay can be used in combination with the RealTi*me* PCR MTB RIF/INH resistance assay.

This study had some limitations that deserve consideration. First, and most important, the number of TB cases in the cohort was low; despite this, we could demonstrate that the systematic use of the Abbott RealTi*me* PCR increased the TB diagnostic yield by nearly 30%. Second, the presence of MTC DNA in PCR-positive/culture-negative specimens was not confirmed by alternative assays. Nevertheless, we are not aware of any molecular assay that outperforms the Abbott RealTi*me* MTB PCR assay in terms of analytical sensitivity; thus, validation attempts by alternative means would have likely failed. Third, the level of culture sensitivity may have been underestimated because of low compliance with the consensus recommendations concerning the number of sputum samples to be collected; and yet, 2 of the 5 PCR-positive/culture-negative patients had 2 sputum samples and 1 additional patient had a bronchiolar lavage (BAL) fluid specimen processed. Fourth, the majority of the patients came from a single tertiary care hospital and about 45% of the patients were hospitalized. Fifth, we did not analyze the health system benefits or overall cost-effectiveness of our proposed diagnostic algorithm because of the low number of TB patients in the cohort and the lack of a consensus method to perform overall cost-effectiveness studies (36).

In summary, to our knowledge, this was the first prospective intervention study to show that universal screening of respiratory specimens from individuals with presumptive TB using a highly sensitive real-time MTC PCR assay improves TB diagnostic accuracy and speed and results in a dramatic reduction in the time to TB treatment for patients with negative AFB smear microscopy results. We propose a novel laboratory diagnostic workflow for TB, but further studies will be required to determine its cost-benefit ratio, how this strategy might affect TB patient clinical outcomes, and if its use would result in significant underdiagnosis of lung NTM disease.

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REFERENCES

1. Greenaway C, Menzies D, Fanning A, Grewal R, Yuan L, FitzGerald JM; Canadian Collaborative Group in nosocomial Transmission of Tuberculosis. 2002. Delay in diagnosis among hospitalized patients with active tuberculosis—predictors and outcomes. Am J Respir Crit Care Med 165:927–933. https://doi.org/10.1164/ajrccm.165.7.2107040.
2. Golub JE, Bur S, Cronin WA, Gange S, Baruch N, Comstock GW, Chaisson RE. 2006. Delayed tuberculosis diagnosis and tuberculosis transmission. Int J Tuberc Lung Dis 10:24–30.
3. Hale YM, Pfyffer GE, Salfinger M. 2001. Laboratory diagnosis of mycobacterial infections: new tools and lessons learned. Clin Infect Dis 33:834–846. https://doi.org/10.1086/322607.
4. Choi HW, Miele K, Dowdy D, Shah M. 2013. Cost-effectiveness of Xpert® MTB/RIF for diagnosing pulmonary tuberculosis in the United States. Int J Tuberc Lung Dis 17:1328–1335. https://doi.org/10.5588/ijtld.13.0095.
5. Diel R, Nienhaus A, Hillemann D, Richter E. 2016. Cost-benefit analysis of Xpert MTB/RIF for tuberculosis suspects in German hospitals. Eur Respir J 47:575–587. https://doi.org/10.1183/13993003.01333-2015.
6. Marks SM, Cronin W, Venkatappa T, Maltas G, Chon S, Sharnprapai S, Gaeddert M, Tapia J, Dorman SE, Etkind S, Crosby C, Blumberg HM, Bernardo J. 2013. The health-system benefits and cost-effectiveness of using Mycobacterium tuberculosis direct nucleic acid amplification testing to diagnose tuberculosis disease in the United States. Clin Infect Dis 57:532–542. https://doi.org/10.1093/cid/cit336.
7. Sohn H, Aero AD, Menzies D, Behr M, Schwartzman K, Alvarez GG, Dan A, McIntosh F, Pai M, Denkinger CM. 2014. Xpert MTB/RIF testing in a low tuberculosis incidence, high-resource setting: limitations in accuracy and clinical impact. Clin Infect Dis 58:970–976. https://doi.org/10.1093/cid/ciu022.
8. Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, Keane J, Lewinsohn DA, Loeffler AM, Mazurek GH, O'Brien RJ, Pai M, Richeldi L, Salfinger M, Shinnick TM, Sterling TR, Warshauer DM, Woods GL. 2017. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis 64:111–115. https://doi.org/10.1093/cid/ciw778.
9. World Health Organization (WHO), Regional Office for Europe. 2017. Algorithm for laboratory diagnosis and treatment-monitoring of pulmonary tuberculosis and drug-resistant tuberculosis using state-of-the-art rapid molecular diagnostic technologies. ISBN 978 92 890 5237 5. WHO, Geneva, Switzerland.
10. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. 2014. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014:CD009593. https://doi.org/10.1002/14651858.CD009593.pub3.
11. World Health Organization. 2013. Global tuberculosis report 2013. WHO/HTM/TB/2013.11. WHO, Geneva, Switzerland.
12. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005–1015. https://doi.org/10.1056/NEJMoa0907847.
13. Marlowe EM, Novak-Weekley SM, Cumpio J, Sharp SE, Momeny MA, Babst A, Carlson JS, Kawamura M, Pandoti M. 2011. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol 49:1621–1623. https://doi.org/10.1128/JCM.02214-10.
14. Luetkemeyer AF, Firnhaber C, Kendall MA, Wu X, Mazurek GH, Benator DA, Arduino R, Fernandez M, Guy E, Metchock B, Sattler F, Teizak E, Wang YF, Weiner M, Swindells S, Sanne IM, Havlir DV, Grinsztejn B, Alland D. 2016. Evaluation of Xpert MTB/RIF versus AFB smear and culture to Identify pulmonary tuberculosis in patients with suspected tuberculosis from low and higher prevalence settings. Clin Infect Dis 62:1081–1088. https://doi.org/10.1093/cid/ciw035.
15. Cowan JF, Chandler AS, Kracen E, Park DR, Wallis CK, Liu E, Song C, Persing DH, Fang FC. 2017. Clinical impact and cost-effectiveness of Xpert MTB/RIF testing in hospitalized patients with presumptive pulmonary tuberculosis in the United States. Clin Infect Dis 64:482–489.
16. Tang N, Frank A, Pahalawatta V, Lampinen J, Coblenz-Korte A, Dunn C, Li C, Cloherty G, Abravaya K, Leckie G. 2015. Analytical and clinical performance of Abbott RealTime MTB, an assay for detection of Mycobacterium tuberculosis in pulmonary specimens. Tuberculosis 95:613–619. https://doi.org/10.1016/j.tube.2015.05.010.
17. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakeore R, Lan NT, Jones-López EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D. 2010. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48:229–237. https://doi.org/10.1128/JCM.01463-09.
18. Chen JH, She KK, Kwong TC, Wong OY, Siu GK, Leung CC, Chang KC, Tam CM, Ho PL, Cheng VC, Yuen KY, Yam WC. 2015. Performance of the new automated Abbott RealTime MTB assay for rapid detection of Mycobacterium tuberculosis complex in respiratory specimens. Eur J Clin Microbiol Infect Dis 34:1827–1832. https://doi.org/10.1007/s10096-015-2419-5.
19. Vinuesa V, Navarro D, Poujois S, Zaragoza S, Borras R. 2016. Performance characteristics of the new Abbott Real Time MTB assay for detection of

Mycobacterium tuberculosis complex in respiratory specimens. Diagn Microbiol Infect Dis 84:212–214. https://doi.org/10.1016/j.diagmicrobio.2015.11.001.
20. Wang SF, Ou XC, Li Q, Zheng QL, Wang YF, Zhao YL. 2016. The Abbott RealTimeMTBassay and the Cepheid GeneXpert assay show comparable performance for the detection of Mycobacterium tuberculosis in sputum specimens. Int J Infect Dis 45:78–80. https://doi.org/10.1016/j.ijid.2016.02.024.
21. Hofmann-Thiel S, Molodtsov N, Antonenka U, Hoffmann H. 2016. Evaluation of the Abbott RealTime MTB and RealTime MTB INH/RIF assays for direct detection of Mycobacterium tuberculosis complex and resistance markers in respiratory and extrapulmonary specimens. J Clin Microbiol 54:3022–3027. https://doi.org/10.1128/JCM.01144-16.
22. Hinić V, Feuz K, Turan S, Berini A, Frei R, Pfeifer K, Goldenberger D. 2017. Clinical evaluation of the Abbott RealTime MTB assay for direct detection of Mycobacterium tuberculosis-complex from respiratory and non-respiratory samples. Tuberculosis 104:65–69. https://doi.org/10.1016/j.tube.2017.03.002.
23. Kostera J, Leckie G, Tang N, Lampinen J, Szostak M, Abravaya K, Wang H. 2016. Analytical and clinical performance characteristics of the Abbott RealTime MTB RIF/INH Resistance, an assay for the detection of rifampicin and isoniazid resistant Mycobacterium tuberculosis in pulmonary specimens. Tuberculosis 101:137–143. https://doi.org/10.1016/j.tube.2016.09.006.
24. González-Martín J, García-García JM, Anibarro L, Vidal R, Esteban J, Blanquer R, Moreno S, Ruiz-Manzano J. 2010. Consensus document on the diagnosis, treatment and prevention of tuberculosis. Arch Bronconeumol 46:255–274. (In Spanish.) https://doi.org/10.1016/j.arbres.2010.02.010.
25. Bemer P1, Palicova F, Rüsch-Gerdes S, Drugeon HB, Pfyffer GE. 2002. Multi-center evaluation of fully automated BACTEC mycobacterial growth indicator tube system MGIT 960 for susceptibility testing of Mycobacterium tuberculosis. J Clin Microbiol 40:150–154. https://doi.org/10.1128/JCM.40.1.150-154.2002.
26. American Thoracic Society; CDC; Infectious Diseases Society of America. 2003. Treatment of tuberculosis. MMWR Recomm Rep 52(RR-11):1–77.
27. CDC. 2012. Report of an expert consultation on the uses of nucleic acid amplification tests for the diagnosis of tuberculosis. https://www.cdc.gov/tb/publications/guidelines/amplification_tests/default.htm.
28. Reid MJ, Shah NS. 2009. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. Lancet Infect Dis 9:173–184. https://doi.org/10.1016/S1473-3099(09)70043-X.
29. Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, Chanda D, Esmail A, Peter J, Dheda K. 2016. Xpert MTB/RIF results in patients with previous tuberculosis: can we distinguish true from false positive results? Clin Infect Dis 62:995–1000. https://doi.org/10.1093/cid/civ1223.
30. Cubero N, Esteban J, Palenque E, Rosell A, Garcia MJ. 2013. Evaluation of the detection of Mycobacterium tuberculosis with metabolic activity in culture-negative human clinical samples. Clin Microbiol Infect 19:273–278. https://doi.org/10.1111/j.1469-0691.2012.03779.x.
31. Scott L, David A, Noble L, Nduna M, Da Silva P, Black A, Venter F, Stevens W. 2017. Performance of the Abbott RealTime MTB and MTB RIF/INH assays in a high TB and HIV coinfection setting in South Africa. J Clin Microbiol 55:2491–2501. https://doi.org/10.1128/JCM.00289-17.
32. Tam KK, Leung KS, To SW, Siu GK, Lau TC, Shek VC, Tse CW, Wong SS, Ho PL, Yam WC. 2017. Direct detection of Mycobacterium tuberculosis and drug resistance in respiratory specimen using Abbott Realtime MTB detection and RIF/INH resistance assay. Diagn Microbiol Infect Dis 89:118–124. https://doi.org/10.1016/j.diagmicrobio.2017.06.018.
33. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponde de Leon A, Daley CL, Samll PM. 1999. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet 353:444–449. https://doi.org/10.1016/S0140-6736(98)03406-0.
34. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP, Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D. 2017. The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. mBio 8:e00812-17. https://doi.org/10.1128/mBio.00812-17.
35. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, Hall SL, Chakravorty S, Cirillo DM, Tukvadze N, Bablishvili N, Stevens W, Scott L, Rodrigues C, Kazi MI, Joloba M, Nakiyingi L, Nicol MP, Ghebrekristos Y, Anyango I, Murithi W, Dietze R, Lyrio Peres R, Skrahina A, Auchynka V, Chopra KK, Hanif M, Liu X, Yuan X, Boehme CC, Ellner JJ, Denkinger CM; study team. 2018. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis 18:76–84. https://doi.org/10.1016/S1473-3099(17)30691-6.
36. Herráez Ó, Asencio-Egea MÁ, Huertas-Vaquero M, Carranza-González R, Castellanos-Monedero J, Franco-Huerta M, Barberá-Farré JR, Tenías-Burillo JM. 2017. Cost-effectiveness study of the microbiological diagnosis of tuberculosis using geneXpert MTB/RIF®. Enferm Infecc Microbiol Clin 35:403–410. https://doi.org/10.1016/j.eimc.2016.06.009.
37. Zwerling A, White RG, Vassall A, Cohen T, Dowdy DW, Houben RM. 2014. Modeling of novel diagnostic strategies for active tuberculosis - a systematic review: current practices and recommendations. PLoS One 9:e110558. https://doi.org/10.1371/journal.pone.0110558.
38. Hughes R, Wonderling D, Li B, Higgins B. 2012. The cost effectiveness of nucleic acid amplification techniques for the diagnosis of tuberculosis. Respir Med 106:300–307. https://doi.org/10.1016/j.rmed.2011.10.005.
39. Aksamit TR, Philley JV, Griffith DE. 2014. Nontuberculous mycobacterial (NTM) lung disease: the top ten essentials. Respir Med 108:417–425. https://doi.org/10.1016/j.rmed.2013.09.014.
40. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Jr, Winthrop K. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–341. https://doi.org/10.1164/rccm.200604-571ST.
41. Honda JR, Knight V, Chan ED. 2015. Pathogenesis and risk factors for nontuberculous mycobacterial lung disease. Clin Chest Med 36:1–11. https://doi.org/10.1016/j.ccm.2014.10.001.
42. Lee MR, Yang CY, Shu CC, Lin CK, Wen YF, Lee SW, Ko JC, Wang JY, Lee LN, Yu CJ. 2015. Factors associated with subsequent nontuberculous mycobacterial lung disease in patients with a single sputum isolate on initial examination. Clin Microbiol Infect 21:250.e1–250.e7. https://doi.org/10.1016/j.cmi.2014.08.025.
43. Lee MR, Yang CY, Chang KP, Keng LT, Yen DH, Wang JY, Wu HD, Lee LN, Yu CJ. 2013. Factors associated with lung function decline in patients with non-tuberculous mycobacterial pulmonary disease. PLoS One 8:e58214. https://doi.org/10.1371/journal.pone.0058214.